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J Mol Biol. 2005 Jul 1;350(1):145-55.

PMID: 15919091 [PubMed - indexed for MEDLINE]

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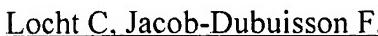
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DN PubMed ID: 15654889

TI Comparison of the substrate specificity of two potyvirus
proteases.

AU Tozser Jozsef; Tropea Joseph E; Cherry Scott; Bagossi Peter;
Copeland

Terry D; Wlodawer Alexander; Waugh David S

CS Department of Biochemistry and Molecular Biology, Research
Center for

Molecular Medicine, University of Debrecen, Debrecen, Hungary..
tozser@indi.biochem.dote.hu

SO The FEBS journal, (2005 Jan) Vol. 272, No. 2, pp. 514-23.
Journal code: 101229646. ISSN: 1742-464X.

CY England: United Kingdom

DT (COMPARATIVE STUDY)

Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200503

ED Entered STN: 19 Jan 2005

Last Updated on STN: 2 Mar 2005

Entered Medline: 1 Mar 2005

AB The substrate specificity of the nuclear inclusion protein a
(NIa)

proteolytic enzymes from two potyviruses, the tobacco etch virus

(TEV) and

tobacco vein mottling virus (TVMV), was compared using
oligopeptide

substrates. Mutations were introduced into TEV protease in an effort to

identify key determinants of substrate specificity. The specificity of

the mutant enzymes was assessed by using peptides with complementary

substitutions. The crystal structure of TEV protease and a homology model of TVMV protease were used to interpret the kinetic data. A comparison of the two structures and the experimental

data suggested that the differences in the specificity of the two enzymes

may be mainly due to the variation in their S4 and S3 binding subsites.

Two key residues predicted to be important for these differences were

replaced in TEV protease with the corresponding residues of TVMV protease.

Kinetic analyses of the mutants confirmed that these residues play a role

in the specificity of the two enzymes. Additional residues in the

substrate-binding subsites of TEV protease were also mutated in an effort

to alter the specificity of the enzyme.

L8 ANSWER 2 OF 2 MEDLINE on STN

DUPPLICATE 2

AN 89370313 MEDLINE

DN PubMed ID: 2475971

TI Characterization of the catalytic residues of the tobacco etch virus

49-kDa proteinase.

AU Dougherty W G; Parks T D; Cary S M; Bazan J F; Fletterick R J
CS Department of Microbiology, Oregon State University, Corvallis
97331-3804.

NC DK39304 (NIDDK)

SO Virology, (1989 Sep) Vol. 172, No. 1, pp. 302-10.
Journal code: 0110674. ISSN: 0042-6822.

CY United States

DT (COMPARATIVE STUDY)

Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

LA English

FS Priority Journals

EM 198909

ED Entered STN: 9 Mar 1990

Last Updated on STN: 3 Feb 1997

Entered Medline: 29 Sep 1989

AB The 49-kDa proteinase of tobacco etch virus (TEV) cleaves the polyprotein

derived from the TEV genomic RNA at five locations. Molecular genetic and

biochemical analyses of the 49-kDa TEV proteinase were performed to test its homology to the cellular trypsin-like serine proteases. A cDNA fragment, containing the TEV 49-kDa proteinase

gene and flanking sequences, was expressed in a cell-free transcription/translation system and resulted in the formation of a

polyprotein precursor that underwent rapid self-processing.

Site-directed

mutagenesis was used to test the effect of altering individual 49-kDa

amino acid residues on proteolysis. The data suggest that the catalytic

triad of the TEV 49-kDa proteinase could be composed of the His234,

Asp269, and Cys339. These findings are consistent with the hypothesis

that the TEV 49-kDa proteinase is structurally similar to the trypsin-like

family of serine proteinases with the substitution of Cys339 as the active

site nucleophile. A structural model of the TEV 49-kDa proteinase

proposes other virus-specific differences in the vicinity of the active

site triad and substrate-binding pocket. The structure may explain the

observed negligible effect of most cellular proteinase inhibitors on the

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Characterization of the catalytic residues of the tobacco etch virus 49-kDa proteinase.

Dougherty WG, Parks TD, Cary SM, Bazan JF, Fletterick RJ.

Department of Microbiology, Oregon State University, Corvallis 97331-3804.

The 49-kDa proteinase of tobacco etch virus (TEV) cleaves the polyprotein derived from the TEV genomic RNA at five locations. Molecular genetic and biochemical analyses of the 49-kDa TEV proteinase were performed to test its homology to the cellular trypsin-like serine proteases. A cDNA fragment, containing the TEV 49-kDa proteinase gene and flanking sequences, was expressed in a cell-free transcription/translation system and resulted in the formation of a polyprotein precursor that underwent rapid self-processing. Site-directed mutagenesis was used to test the effect of altering individual 49-kDa amino acid residues on proteolysis. The data suggest that the catalytic triad of the TEV 49-kDa proteinase could be composed of the His234, Asp269, and Cys339. These findings are consistent with the hypothesis that the TEV 49-kDa proteinase is structurally similar to the trypsin-like family of serine proteinases with the substitution of Cys339 as the active site nucleophile. A structural model of the TEV 49-kDa proteinase proposes other virus-specific differences in the vicinity of the active site triad and substrate-binding pocket. The structure may explain the observed negligible effect of most cellular proteinase inhibitors on the activity of this viral proteinase.

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Molecular genetic and biochemical evidence for the involvement of the heptapeptide cleavage sequence in determining the reaction profile at two tobacco etch virus cleavage sites in cell-free assays. [Virology. 1989]

Identification of essential amino acid residues in the functional activity of poliovirus 3A protein. [Virology. 1991]

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Mutational analysis of the tobacco etch potyviral 35-kDa proteinase: identification of essential residues and requirements for autoproteolysis. [Virology. 1992]

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